This application is a divisional application of application Serial No. 08/106,775, filed August 16, 1993, abandoned, which is a continuation application of Serial No. 07/780,847, filed October 23, 1991, abandoned, which is a continuation application of Serial No. 07/304,281, filed January 31, 1989, abandoned.

Page 3, line 25, delete "FGF-5";
Page 4, line 16, delete "cells" and substitute therefor --tissue--;

Page 6, line 14, delete "FGF-5";

Page 12, line 2, delete the second recitation of "KGF or";

Page 13, line 1, delete "I-"; delete "heparin-Sepharose...NaCl." and insert the following:

--Heparin-Sepharose affinity chromatography of conditioned medium from M426 human embryonic fibroblasts. Approximately 150 ml of ultrafiltration retentate derived from five liters of M426 conditioned medium were loaded onto a heparin-Sepharose column (6 ml bed volume) in 1 hr. After washing the column with 150 ml of the equilibration buffer, 20 mM Tris-HCl, pH 7.50/0.3M NaCl, the retained protein (<5% of the total protein in the retentate) was eluted with a modified linear gradient of increasing NaCl concentration. Fraction size was 3.8 ml and flow rate during gradient elution was 108 ml/hr. Two μl of the indicated fractions were transferred to microtiter wells containing a final volume of 0.2 ml for assay of ^{3}H-thymidine incorporation in BALB/MK cells as described in the methods.--

At page 13, line 8, delete "I-" and insert --A, 2B and 2C--;
line 10, delete "and" and substitute therefor --an--;
at lines 11-22, delete everything after "the profile on" and insert the following:

--Reversed-phase C_4HPLC of BALB/MK mitogenic activity. Active fractions eluted from heparin-Sepharose with 0.6M NaCl were processed with the Centricon-10 and loaded directly onto a C_4 Vydac column (4.6 X 250 mm) which had been equilibrated in

0.1% trifluoroacetic acid/20% acetronitrile (ACN). After washing the column with 4 ml of equilibration buffer, the sample was eluted with a modified

linear gradient of increasing % ACN. Fraction size was 0.2 ml and flow rate was 0.5 ml/min. Aliquots for the assay of ^{3}H-thymidine incorporation in BALB/MK cells were promptly diluted 10- fold with 50µg/ml bovine serum albumin/20 mM Tris-HCl, pH 7.5, and tested at a final dilution of 200-fold. (B) NaDodSO$_4$/PAGE analysis of selected fractions from the C_4 chromatography shown in panel A. Half of each fraction was dried, redissolved in NaDodSO$_4$/2-mercaptoethanol, heat denatured and electrophoresed in a 14% polyacrylamide gel which was subsequently stained with silver. The position of each molecular weight marker (mass in kDa) is indicated by an arrow. (C) DNA synthesis in BALB/MK cells triggered by the fractions analyzed in Panel B. Activity is expressed as the fold stimulation over background which was 100 cpm.--

line 23, delete "I-";

line 24 to page 14, line 2, delete "sieving chromatography... bioassay." and insert the following:

--Molecular sieving HPLC (TSK 3000SW) chromatography of the BALB/MK mitogenic activity. Approximately 50 µl of a Centricon-processed, 0.6M NaCl pool from HSAC were loaded onto a LKB GlasPac TSK G3000SW column (8 X 300 mm), previously equilibrated in 20 mM Tris-HCl, pH 6.8/0.5M NaCl, and eluted as 0.2 ml fractions at a flow rate of 0.4 ml/min. Aliquots of 2 µl were transferred to microtiter wells, containing a final volume of 0.2 ml for assay of ^{3}H-thymidine incorporation in BALB/MK cells. The elution positions of molecular weight markers (mass in kDa) were as indicated by the arrows.—

At page 14, line 3, delete "I-"; also at line 3, delete "comparison of...factors." and insert the following:

--Comparison of BALB/MK DNA synthesis in response to TSK-purified mitogen and other growth factors. Incorporation of ^{3}H-thymidine into trichloracetic acid-insoluble DNA, expressed as fold stimulation over background, was measured as a function of the concentration of the indicated growth factors. Background values with no sample added were 150 cpm. The results represent mean values of two independent experiments. Replicates in each experiment were within 10% of mean values. TSK-purified mitogen, °_____°; EGF, ^_____^; aFGF, _____; bFGF, °_____°.--

line 6, delete "I-"; also at line 6, delete "comparisons-of growth...factors." and insert the following:

-- Comparative growth of BALB/MK cells in a chemically defined medium in response to different combinations of growth factors. Cultures were plated at a density of 2.5 X 10^{4} cells per dish on 35 mm Petri dishes precoated with poly-D-lysine/fibronectin in a 1:1 mixture of Eagle's minimal essential medium and Ham's F12 medium supplemented with transferrin, $Na^{2}SeO^{3}$, ethanolamine and the growth factors indicated below. After 10 days, the plates were fixed and stained with Giemsa. Key: a) no growth factor; b) EGF alone; c) insulin alone; d) KGF alone; e) EGF and dialyzed fetal calf serum (final concentration, 10%); f) KGF and EGF; g) KGF and insulin: h) EGF and insulin. Final concentrations of the growth factors were as follows: EGF, 20 ng/ml; insulin, 10μg/ml; and KGF, 40 ng/ml.--

At page 14, delete lines 10-28 and at page 15, delete lines 1-3 and insert therefor:

--Fig. 6 outlines a schematic representation of human KGF cDNA clones. Overlapping pCEV9 clones 32 and 49, used in sequence determination, are shown above a diagram of the complete structure in which untranslated regions are depicted by a line and the coding sequence is boxed. The hatched region denotes sequence of the signal peptide. Selected restriction sites are indicated.

Fig. 7 documents the KGF cDNA nucleotide and predicted amino acid sequences. Nucleotides are numbered on the left; amino acids are numbered throughout. The N-terminal peptide sequence derived from purified KGF is underlined. The hydrophobic N-terminal domain is italicized. The potential asparagine-linked glycosylation site is overlined. The variant polyadenylation signals, AATTAA and AATACA, close to the 3' end of the RNA, are boxed.

Fig. 8 shows identification of KGF mRNAs by Northern blot analysis. Lanes a and c, poly(A)-selected M426 RNA; lanes b and d, total cellular M426 RNA. Filters were hybridized with a ^{32}P-labeled 695 bp *Bam*HI/*Bcl*I fragment from clone 32 (Probe A, Fig. 6), lanes a and b, or a 541 bp *Apa*I/*Eco*RI fragment from clone 49 (Probe B, Fig. 6), lanes c and d.--.

At page 15, line 4, delete "II-2" and insert --9--;

line 7, after "homology" insert --(shaded boxes)--; line 8, after "sequences" insert --(hatched boxes)--;

line 9, after "residues" insert --(positions labeled with a "C")--.

Delete lines 10-18 and insert the following:

-- Fig. 10 shows northern blot analysis of KGF mRNA in normal human cell lines and tissues, and comparison with mRNA expression of other growth factors with known activity on epithelial cells. Total cellular RNAs were isolated by cesium trifluoro-acetate gradient centrifugation. 10μg of RNA were denatured and electrophoresed in 1% formaldehyde gels. Following milk alkali denaturation (50 mM NaOH for 30'), RNA was transferred to nitrocellulose filters using 1M ammonium acetate as a convectant. Filters were hybridized to a 32^{P}-labeled cDNA probe containing the 647 bp EcoRI fragment from the 5' end of the KGF coding sequence (A) or similar probes from the other growth factor DNAs. The following human cell types were used: squamous cell carcinomas (A253, A388 and A431); mammary epithelial cells B5/589; immortalized bronchial epithelial cells (S6 and R1); keratinocytes immortalized with Ad12-SV40;

primary human keratinocytes; neonatal foreskin fibroblasts, (AG1523) adult skin fibroblasts (501T); and embryonic lung fibroblasts (WI-38 and M426) and tissues, revealing that a single 2.4 kb transcript was present in RNA form human embryonic fibroblasts and from adult skin fibroblasts, while no transcript was detected in the (B5/589) epithelial or (HA 83) glial cell lines or in primary cultures of human saphenous vein endothelial cells. --

Page 15, delete lines 19-28.

Page 18, line 15, delete "I-".

Page 20, line 13, delete "I-".

Page 22, line 9, delete "II-1A" and insert --6--;

line 15, delete "II-B" and insert --7--;

line 22, delete "FGF-5".

Page 24, line 27, delete "46" and substitute therefor --about 40--.

Page 25, line 2, delete "II-1" and insert --7--;

line 3, delete "116" and substitute therefor "about 140";

line 8, delete "its activity is enhanced by" and substitute therefor --lacks susceptibility to--.

Page 26, line 8, delete "NH_2" and insert --N--.

Page 30, lines 17, delete "plus an R at its C-terminus" and insert--45--;

line 19, delete "II-1" and insert --7--.

Page 41, line 14, delete "I-".

Page 42, line 10, delete "I-2" and insert --2A--;

line 15, delete "I-";

line 16, delete "I-";

line 24, delete "I-";

line 28, delete "I-";

Page 43, line 9, after "." insert the following:

--Table 1. Growth Factor Purification

Purification Step	Protein (mg)	Total activity (units)*	Specific activity (units/mg)
Conditioned medium (10 liters)	1.4×10^{3a}	2.5×10^{4}	1.8×10^{1}
Ultrafiltration (retentate)	1.3×10^{3a}	3.2×10^{4}	2.5×10^{1}
HSAC	0.73^{b}	1.6×10^{4}	2.2×10^{4}
0.6 MM NaCl pool			
TSK-G3000 SW	8.4×10^{-3b}	2.7×10^{3}	3.2×10^{5}
C_4-HPLC	6.1×10^{-3b}	2.1×10^{2}	3.4×10^{4}

Recoveries were calculated by assuming that all of the mitogenic activity in the starting material was due to the isolated factor.

One unit of activity is defined as half of the maximal stimulation of thymidine incorporation induced by TSK-purified factor in the BALB/MK bioassay, in which approximately 3 ng of the TSK-purified factor stimulated 1 unit of activity.

[a]Protein was estimated by using the Bradford reagent from BioRad (Bradford, M., 1976, Anal. Biochem. 72. 248-254).

[b] Protein was estimated by using $A^{1\%}_{214} = 140$.--

At page 49, line 17 to page 52, line 24, delete all.

At page 43, line 13, delete "I-" and insert --A-2C--;

line 18, delete "I-";

Page 44, line 6, delete "I-".

line 16, delete "I-";

line 22, delete "I-";

Page 45, line 8, delete "I-";

line 18, delete "I-";

line 23, after "." insert the following:
--Table 2. Target Cell Specificity of Growth Factors

Growth Factor	Epithelial			Fibroblast	Endothelial
	BALB/MK	B/589	CCL208	NIH/3T3S	Human saphenous vein
KGF	500-1000	2-3	5-10	<1	<1
EGF	100-200	20-40	10-30	10-20	n.d.
TGFa	150-300	n.d.	n.d.	10-20	n.d.
AFGF*	300-500	2-3	5-10	50-70	5
bFGf	100-200	2-3	2-5	50-70	5

Comparison of maximal thymidine incorporation stimulated by KGF and other growth factors in a variety of cell lines, expressed as fold stimulation over background.

This data represents a summary of four different experiments.
*Maximal stimulation by aFGF required the presence of heparin (Sigma), 20 μg/ml.
n.d. = not determined--

Page 53, line 14, delete "NH_2" and insert --N--.

line 21, delete "FGF-5".

Page 55, line 16, delete "II-1" and insert --7--;

Page 56, line 3, delete "the conditioned medium of human--fibroblast" and substitute therefor --human epithelial--.

Page 58, lines 9-10, delete "reference II-3" and insert --Rubin *et al., Proc. Natl. Acad. Sci. USA* 86: 802-806 (1989)--.

Page 59, line 13, delete "II-1A" and insert --6--;
line 23, delete "II-1B" and insert --7--;

Page 60, line 14, delete "NH_2" and insert --N--.

Page 61, line 1, delete "FGF-5";

line 7, delete "II-1A" and insert --6--;

line 9, delete "II-1C" and insert --8--;

line 17, delete "II-1A" and insert --6--;

line 18, delete "II-1C" and insert --8--;

line 27, delete "II-3" and insert --10--;

Page 62, line 19, delete "II-3" and insert --10--;

line 27, delete "II-3" and insert --10--.

Page 63, line 5, delete "II-21, II-22" and insert: -- Schreiber *et al., Proc. Natl. Acad. Sci. USA* 82: 6138-6142 (1985), Gospodarwizc *et al., J. Cell Physiol.* 128: 475-485 (1986)--;

line 8, delete "II-22" and insert--Gospodarwizc *et al., supra.*--

line 10, delete "II-3" and insert --10--;

line 15, after "." insert the following:

--TABLE 3. Effect of Heparin on KGF Mitogenic Activity.

Growth Factor	BALB/MK		NIH/3T3	
	-	+	-	+
KGF	150	9.5	<1	<1
AFGF	106	259	10.4	68
BFGF	30	124	45.7	70

Cells were plated in microtiter plates, grown to confluence in serum containing media and then placed in a serum-free medium for 24-72 hr prior to sample addition. Mitogenesis assays were performed as described (see Experimental Section I, above and Rubin et al. Proc. Natl. Acad. Sci. USA 86: 802-806 (1989). Where indicated, heparin was included in the culture media at a final concentration of 20 μg/ml. The concentration of all the growth factors was 50 ng/ml. The results represent fold stimulation of ^{3}H-thymidine incorporation in the indicated assay cell in the presence (+) or absence (-) of heparin. Each value represents the mean result from two independent experiments in which each point, in turn, represents the mean value of duplicate analyses.--

Page 64, line 14, delete "II-1" and insert --7--;

line 18-19, delete "was not identified" and substitute therefor --appeared to be arg--.

Page 67, line 19, delete "*Sau*I" and substitute therefor --*Sau*3AI--;

line 22, delete "II-1" and insert --7--;

line 24, delete "37 (Phe), 38 (leu) and 39 (arg)" and substitute therefor --39(arg) and 40--.

Page 70, line 5, delete "FGF-5 and hst are transforming genes originally detected by DNA-mediated gene transfer" and substitute therefor:

--The hst gene was identified as a transforming gene from a human stomach tumor (Taira *et al., Proc. Natl. Acad. Sci. USA* 84: 2980-2984(1987), adjacent normal stomach tissue (Yoshida *et al., Proc. Natl. Acad. Sci. USA* 84: 7305-7309 (1987), and from Kaposi's sarcoma (Delli-Bovi *et al., Proc. Natl. Acad. Sci. USA* 84: 5660-5664 (1987), by standard NIH/3T3 transfection assays--.

Page 71, line 22, delete "II-2" and insert --9--.

Page 72, line 9, delete "II-2" and insert --9--;

line 11, delete "FGF-5";

line 16, delete "II-1B" and insert --7--;

line 26, delete "II-2" and insert --9--.

Page 74, line 23, delete "II-3" and insert --Rubin *et al., Proc. Natl. Acad. Sci. USA* 86: 802-806 (1989)--.

Page 75, line 20 to page 79, line 4, delete all.

Page 87, line 1, delete "I-" and then move entire page, as amended, to page 43, above line 10.

Page 88, line 1, delete "I-" and then move entire page to page 45, above line 24.

Page 89, line 1, delete "I-" and then move entire figure legend, as amended, to page 13, line 2, after "results of".

Page 90, top of page, delete "I-" and delete "SEE LEGEND NEXT PAGE".

Page 91, delete "Figure I-2. (A), and then move entire page, as amended, to page 13, line 11, after "on".

Page 92, delete "Figure I-3." and then move entire figure legend to page 14, above line 3.

Page 93, delete "Figure I-4." and then move entire figure legend, as amended, to page 14, line 3, after "illustrates a".

Page 94, delete "Figure I-5." and then move entire figure legend, as amended, to page 14, line 6, after "shows".

Page 95, line 1, delete "II-1" and insert –3 --and amend Table by substituting "-" for "+" and "+" for "-" in the first line of the Table, as indicated;

line 5, delete "II-3" and insert -- Rubin et al. Proc. Natl., Acad. Scie. USA 86: 802-806 (1989)--, then move entire page to page 63, line 15, after "treatment.".

Page 96, delete "II-1A. SEE LEGEND FOLLOWING" and insert --6--.

Page 97, delete "II-1B. SEE LEGEND FOLLOWING" and insert --7--.

Page 98, delete "II-1C. SEE LEGEND FOLLOWING" and insert --8--.

Page 99, line 1, delete "Figure II-1. Nucleotide sequence and deduced amino acid sequence of KGF cDNA, and identification of KGF gene transcripts."

line 3, delete "(A)" and substitute therefor --Figure 6 outlines a--;

line 12, delete "(B)" and substitute therefor --Figure 7 documents the--;

line 22, delete "(C)" and substitute therefor --Figure 8 shows--;

line 27, delete "II-1A" after "Fig.".

Page 100, line 2, delete "II-1A" after "Fig" and insert –6--.

Move entire text of pages 99 and 100, as amended, to page 14, line 10, before "Table".

Page 101, delete lines 1-6, which is a figure legend.

Page 102, top of page, delete "II-3. SEE LEGEND NEXT PAGE" and insert --10--.